IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
COX et al.
Serial No.: 09/706,243 Art Unit: 1631
Filing Date: November 3, 2000 Examiner: J. Brusca

Title: REGULATION OF ENDOGENOUS GENE EXPRESSION USING ZINC FINGER PROTEINS

DECLARATION PURSUANT TO 37 C.F.R. § 1.132 OF CARL PABO, Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Carl Pabo, hereby declare as follows:

1. I received my Ph.D. in Biochemistry and Molecular Biology from Harvard University in 1980. I am currently Senior Vice President and Chief Scientific Officer at Sangamo BioSciences, Inc. and have been at Sangamo since 2001. Before joining Sangamo, I was an Investigator of the Howard Hughes Medical Institute and a Full Professor at the Massachusetts Institute of Technology since 1991. Based on my accomplishments, I have been elected to the National Academy of Sciences and the American Academy of Arts and Sciences. A copy of my Curriculum Vitae (Exhibit A) is attached hereto.

2. I am extremely familiar with studies of both zinc finger proteins and protein delivery having actively studied, worked and published in this discipline for over 10 years. I have co-authored numerous publications and patents in the field of gene expression, including publications relating to zinc finger proteins. I have also co-authored numerous publications and patents in the field of protein delivery. (See, *e.g.,* U.S. Patent Nos. 6,316,003; 5,804,604; 5,747,641; and 5,674,980).

3. I have reviewed pending Patent Application Serial No. 09/706,243 for "REGULATION OF ENDOGENOUS GENE EXPRESSION USING ZINC FINGER PROTEINS" by Cox et al., (hereinafter "the specification") and the currently pending claims. I have also reviewed the Office Action dated August 6, 2002. Therefore, I am familiar with the issues raised by the Examiner in the Office Action.

4. I understand that the pending claims are directed to methods of modulating (activating or inhibiting) expression of an endogenous gene by introducing an engineered zinc finger protein into a cell. It is further my understanding that the claims encompass methods in which the zinc finger protein is introduced into the cell as a protein.

5. It is my opinion that, as a technical matter, a skilled worker could have readily practiced the methods of the pending claims in light of the specification, together with the common general knowledge, tools and methods available as of the effective filing date of January 1999. I base this opinion on the facts set forth below; however, I call attention to the fact that introducing proteins into cells would not have required undue experimentation and, once introduced into the cell, these proteins would have been expected to modulate expression of endogenous genes. In addition, in drawing my conclusions, I have considered the nature of the claims, the quantity of experimentation required to practice the subject matter of the claims, the direction present in the specification, the state of the field at the time the application was filed and the level of skill in the art.

6. At the outset, I note that the term "skilled worker" with a routine level of skill in the field of molecular biology in January 1999 had a Ph.D. degree and two or more years of post-doctoral training.

7. In January 1999, the quantity of experimentation required to identify protein delivery vehicles suitable for introducing zinc finger proteins into cells was quite low. For example, membrane translocation peptides, toxins, liposomes, antibodies and other moieties had been used to insert proteins into the cells for some time, as described for example, on pages 43-47 of the specification, including the references cited in these pages. Based on these extensive teachings, it is evident that a skilled worker would have easily recognized that the protein delivery moieties described in the specification and known in the field could be used to deliver an engineered ZFP to a cell. Thus, it is my opinion that it would have required only routine experimentation to select a protein delivery vehicle that would introduce a functional engineered zinc finger protein into a cell, as claimed.

8. In addition, a skilled worker could have easily tested and used any protein delivery vehicle for its ability to deliver zinc finger proteins. As noted above, polypeptides were routinely administered by a variety of methods known at the time of filing. For example, administration of proteins to cells generally is described in Debs et al. (1990) J. Biol. Chem. 265:10189-10192 (Exhibit B) and Phelan et al. (1998) Nat Biotechnol 16(5):440-443 (Exhibit C). These and other references address the pertinent question at issue here -- whether delivery of proteins to cells was unpredictable as of January 1999 in view of the teachings of the specification and state of the field. These references plainly confirm that protein delivery vehicles described in the specification had been successfully employed thereby confirming that

protein delivery as a whole was **not** unpredictable as of January 1999. Debs, for instance, establishes that the skilled worker could introduce a transcription factor protein into cultured cells, and that the introduced transcription factor is functional in the cell. Similarly, Phelan establishes that functional p53 can be delivered into cells using VP22 as a delivery vehicle (see, also, page 44, line 23 of the specification describing the use of VP22 as a protein delivery vehicle):

> We show that chimeric polypeptides, consisting of VP22 linked to the entire p53 protein, retain their ability to spread between cells and accumulate in recipient cell nuclei. Furthermore the p53-VP22 chimeric protein efficiently induces apoptosis in p53 negative human osteosarcoma cells resulting in a widespread cytotoxic effect. ... These results demonstrating intracellular transport of large functional proteins, indicate that VP22 delivery may have applications in gene therapy. (Phelan, Abstract).

These references are clearly representative of the high level of existing skill in the art and the fact that delivery of proteins to cells was considered predictable in January 1999. Many other examples of protein delivery modes were also known at the time, including patents on which I am a co-inventor, examples of which are listed in paragraph 2 above. Moreover, the disclosure of the specification includes a myriad of protein delivery vehicles and also provides significant direction of how to use these delivery vehicles to introduce engineered zinc finger proteins, for example on pages 43-47 of the specification. In sum, to the skilled worker, administering the claimed engineered zinc finger proteins to a cell would have been routine and would have required little or no experimentation.

9. Furthermore, the specification provides significant direction for evaluating whether a particular protein delivery vehicle targeted a given cell type and whether the protein delivered by the vehicle functioned in the cell after introduction. Those of us working in the field of zinc finger proteins are well versed in the various tests for determining whether zinc finger proteins are inserted into and are functional in a cell, for example, by assays described on pages 36-38 of the specification. Examples present in the specification demonstrate such assays. (See, Examples IV, V, and VI). Furthermore, preparing protein delivery vehicles in January of 1999 was well within the purview of a skilled worker. Even if a particular delivery vehicle were inoperable for some reason, the skilled worker would have readily selected alternatives known at the time and described in the specification.

10. Finally, there is no question that the specification as filed describes methods in which the zinc finger proteins are introduced into cells as nucleic acids, are then expressed in the cells and act to modulate endogenous gene expression. In light of the significant direction

present in the specification and the state of the field at the time the application was filed regarding protein delivery, it is my opinion that any skilled worker would have recognized that once a zinc finger protein was delivered to the cell using routine protein delivery mechanisms, the zinc finger protein would function as described in the specification and modulate expression of endogenous genes.

11. In view of the foregoing facts regarding the routine nature of experimentation required to make, use and deliver functional proteins such as engineered zinc finger proteins, the extensive direction provided by the specification, the straightforward nature of the claimed subject matter, the high level of the skilled worker, the sophistication of the art, and the predictability of the art, it is my unequivocal opinion that the specification enabled, in January 1999, a skilled worker to practice the methods as claimed.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1/29/03
Date

Carl O Pabo
Carl Pabo, Ph.D.